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EDITORIAL COMMENT

Tracking Cell Therapy

Bioluminescence Lighting the Way*

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Cell-based therapies involving various types of stem cells, pancreatic β-cell islets, and bone marrow–derived mononuclear cells (MNCs), among others, are thought to have great potential as treatments for a number of diseases, such as cancer, heart disease, and diabetes. Peripheral artery disease is caused by reduced blood flow to the limbs in arteries that have become occluded by atherosclerotic plaques. Despite approaches such as stent emplacement or vascular bypasses, a number of patients suffering from peripheral artery disease do not recover and

See page 46

may have to undergo amputation (1). Due to the lack of an effective treatment and the dire outcomes, therapeutic angiogenesis using gene therapy approaches were explored and offered some potential for rescuing ischemic limbs. However, after promising pre-clinical experiments, most clinical trials failed. Most recently, a large trial of fibroblast growth factor gene transfer did not alter the outcome of keeping patients amputation-free (2). MNC transplantation has been proposed as an alternative therapy for sufferers of this disease due to the roles of these cells identified in the natural response to ischemia (3). In addition, the MNCs of peripheral artery disease sufferers are relatively few in number and have been shown to function poorly (4), further motivating MNC transplantation. The results of clinical trials using MNCs in peripheral artery disease have been positive (1), motivating the use of imaging-based methods of cell tracking to facilitate optimization of MNC therapeutic strategies.

The development of cell tracking. Soon after cell-based therapies were proposed, it was recognized that tracking the transplanted cells via imaging would be highly valuable for technique optimization. Cell tracking is often enabled by labeling the donor cells with a diagnostically active substance, such as iron oxides for magnetic resonance imaging (5), indium-111 for single-photon emission computed tomography (6), or quantum dots for fluorescence imaging techniques (7). Cell tracking offers the possibility to determine the site of injection of the cells, their migration, and, it was thought, the residence time of cells in the diseased tissue. However, the interpretation of cell tracking images has been hampered by the fact that when the transplanted cells die, macrophages or other neighboring cells often take up the labeling material and the signal remains in the tissue, yielding a false-positive result that the transplanted cells are still resident in the tissue (8).

Bioluminescence in cell tracking. In order to address the issue of false-positive signals arising from the use of exogenous labels, some have turned to transplanting bioluminescent cells in order to measure viability (9). The methodology to make the cells bioluminescent is normally to transfect the cells of the donor ex vivo with viral constructs, then transplant them into the recipient. However, questions surround the effect of transfecting the donor cells, such as whether perturbation of the cells' genetic profile occurs and the impact of injecting the recipient with viral material. In this issue of *iJACC*, van der Bogt et al. (10) report the use of MNCs from donor mice that constitutively express both luciferase and green fluorescent protein

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(GFP), therefore avoiding some of the issues surrounding the use of viruses or other gene transfer techniques.

The authors transplanted MNCs into wild-type mice of the same background that had undergone unilateral femoral artery ligation via electrocoagulation. In vitro experiments had confirmed the extracted cells to express luciferase and GFP. Strong signals were seen in the first few days post-injection in the wounded leg from in vivo bioluminescence measurements, when the MNCs were injected intramuscularly. By the 13th day post-injection, however, the majority of the transplanted MNCs had died, and essentially all MNCs had died by 27 days. This result was confirmed by immunohistological staining for GFP, when very few MNCs were found in sections of the injured muscle. Laser Doppler perfusion imaging of the mouse paws was used as a measure of recovery of blood flow. The cell treatments did not result in a significantly faster or more substantial recovery than control, with blood flow returning to pre-ligation levels after 14 days. Histological detection of the number of collateral arteries confirmed that there were no therapeutic advantages derived from the MNCs.

In addition to intramuscular injection, MNCs were injected intravascularly to determine the effect of different injection routes. Bioluminescence imaging showed the MNCs in this case to home to several sites: the wounded leg, liver, spleen, and bone marrow. In this experiment, it was also the case that the blood flow recovery in the paws was unaffected by the MNC treatment. The cell tracking imaging results reported seem to correspond with the therapeutic measurements—that is, the poor MNC survival observed from bioluminescence imaging resulted in no improvement in blood flow recovery compared with controls. This demonstrates the potential of bioluminescence-based tracking for optimization or screening of cell therapies.

That no therapeutic effect of MNC transplantation was observed is discouraging for this field. Nevertheless, it is important to note that the model used in this study was an acute injury model, as opposed to the chronic state of peripheral artery disease for which this therapy has been proposed. The MNCs of patients with advanced atherosclerotic peripheral artery disease have been shown to have reduced ability to regenerate blood flow (4), which will not be the case for healthy young mice, as shown by the recovery of blood flow in 14 days in untreated mice. Nevertheless, this study points to the need for strategies to optimize therapeutic response, notably the need for methods to prolong cell survival, such as coadministration of drugs. Other routes to protocol optimization might be to improve cell homing to the diseased tissue or to identify or isolate subpopulations of cells that will have enhanced therapeutic effect. However, if other studies reveal similar findings, MNCs may have no role in therapeutic angiogenesis.

The future for bioluminescence in cell tracking. The results reported by van der Bogt et al. (10) show bioluminescence imaging to be a powerful technique for cell tracking in small animals such as mice. Methods such as this, or reporter gene methods, to measure the viability of transplanted cells will be vital for the development of successful cell therapies. It is unlikely, however, that bioluminescence will be translated to patients due to the poor tissue penetration of light and concerns surrounding immunogenicity of luciferase and injections of luciferin (11). Cell tracking in patients will likely be pursued with techniques that have much better tissue penetration, such as nuclear techniques or magnetic resonance imaging, both of which have already been studied in patients (5). Nevertheless, bioluminescence imaging will be invaluable in refining cell therapy methodology in pre-clinical settings before moving on to clinical trials.

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JACC: CARDIOVASCULAR IMAGING, VOL. 5, NO. 1, 2012
JANUARY 2012:56–8

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